IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:	Group Art Unit: 1647
COX	Examiner: Stoica, Elly Gerald
Serial No.: 10/774,149	Confirmation No.: 7193
Filed: February 5, 2004	DECLARATION OF GEORGE COX (Under 37 CFR 1.132)
Atty. File No.: 4152-1-PUS-5	
For: "CYSTEINE VARIANTS OF GRANULOCYTE COLONY-STIMULATING FACTOR"	***Via Electronic Filing***

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Dear Sir:

I, George Cox, declare as follows:

1. I am the sole inventor of the above-identified application and am familiar with the application. I am a skilled artisan in the fields of molecular and cellular biology and have been involved with the experiments described in paragraphs 4-5 below.

2. This Declaration is being submitted in conjunction with an Amendment and Response to an Office Action having a mailing date of August 30, 2006.

3. The following discussion is provided in response to the Examiner's rejections of Claims 24-52 under 35 U.S.C. § 112, first paragraph. Specifically, the data presented in the following paragraphs demonstrate that I and my colleagues have constructed two cysteine muteins of granulocyte colony-stimulating factor (G-CSF) that fall within the scope of the present claims, and have shown that the muteins are biologically active in an *in vitro* cell-based proliferation assay for G-CSF activity. Moreover, the following data demonstrate that modification of the free cysteine moieties on the muteins with polyethylene glycol (PEG) does not inhibit the biological activity of the muteins.

4. Using the methods and techniques described in Examples 1 and 5 of the above-referenced application, muteins containing a single cysteine substitution were constructed in the human G-CSF gene and were expressed in an *E. coli* expression system. The muteins were constructed in a G-CSF protein in which cysteine-17 of G-CSF was changed to serine (C17S).

The reference to position numbers is made with regard to SEQ ID NO:6 of the specification, which represents the amino acid sequence of the mature G-CSF protein (see Example 5). The resulting muteins include insertions preceding the first amino acid of the mature protein (referred to as *-1C) and following the last amino acid of the mature protein (referred to as *-175C).

5. The muteins described in paragraph 4 above were expressed in *E. coli* as periplasmically secreted proteins using the *E. coli* STII signal sequence, and tested for biological activity *vs.* a wild type G-CSF control (obtained from R&D Systems, Inc., Minneapolis, MN) and a G-CSF (C17S) control protein prepared in my laboratory in an *in vitro* cell-line based proliferation assay. The two G-CSF cysteine muteins (*-1C and *175C) described in paragraph 4 were purified to homogeneity. The *-1C and *175C muteins were modified with polyethylene glycol ("PEGylated") using techniques described in Example 1 of the above-identified application. The PEGylated forms of the *-1C and *175C cysteine muteins were purified away from any unmodified material. The purified cysteine muteins and the purified PEGylated cysteine muteins were assayed for biological activity *vs.* a wild type G-CSF and G-CSF (C17S) in an *in vitro* cell-line proliferation assay using the murine NFS-60 cell line. All of the purified cysteine muteins and the purified PEGylated cysteine muteins were biologically active. The EC_{50} of the purified cysteine muteins and the purified PEGylated cysteine muteins ranged from indistinguishable from the EC_{50} of each of the wild type G-CSF and G-CSF (C17S) controls to within approximately 2-fold of the EC_{50} of each of the wild type G-CSF and G-CSF (C17S) controls.

6. I hereby declare that all statements made herein of my own are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing therefrom.

Date: November 28, 2006 By: George Cox

George Cox